

Readme Document regarding data repository for Royal Society Proceedings B paper as follows.

Title: Neural control of tuneable skin iridescence in squid

Authors: Wardill TJ*¹, Gonzalez-Bellido PT*¹, Crook RJ^{1, 2}, Hanlon RT¹.

*shared first authorship

Corresponding Author: twardill@mbi.edu & paloma@mbi.edu

¹ Program in Sensory Physiology and Behaviour, Marine Biological Laboratory, 7 MBL St, Woods Hole, MA 02543, USA.

² Department of Integrative Biology and Pharmacology, University of Texas Medical School at Houston, 6431 Fannin Street, Houston, TX 77030, USA.

In addition to the **Introduction**, find also the **Materials and Methods** document that describes the methods used to collect the data for this paper. The final readme, **File Descriptions**, describes how the files are arranged in various Zip files. The data within these zip files should be considered the gold standard data, although considerably more data exists than is reported in this repository. Please contact the authors directly using the contact details above for any additional data.

Abstract: Fast dynamic control of skin coloration is rare in the animal kingdom, whether it be pigmentary or structural. Iridescent structural coloration results when nanoscale structures disrupt incident light and selectively reflect specific colours. Unlike animals with fixed iridescent coloration (e.g. butterflies), squid iridophores (i.e. aggregations of iridescent cells in the skin), produce dynamically tuneable structural coloration, as exogenous application of acetylcholine (ACh) changes the colour and brightness output. Previous efforts to stimulate iridophores neurally or to identify the source of endogenous ACh were unsuccessful, leaving researchers to question the activation mechanism. We developed a novel neurophysiological preparation in the squid *Doryteuthis pealeii* and demonstrated that electrical stimulation of neurons in the skin shifts the spectral peak of the reflected light to shorter wavelengths (>145 nm) and increases the peak reflectance (>245 %) of innervated iridophores. We show ACh is released within the iridophore layer and that extensive nerve branching is seen within the iridophore. The dynamic colour shift is significantly faster (17 s) than the peak reflectance increase (32 s) revealing two distinct mechanisms. Responses from a structurally altered preparation indicate that the reflectin protein condensation mechanism explains peak reflectance change, while an undiscovered mechanism causes the fast colour shift.

Key index words: Structural coloration, neural stimulation, skin patterning.

Sponsors: We thank fellow lab members for their support and discussion of this study, and in particular Lydia Mäthger for help with spectrometry and advice and Tom Cronin for comments on the manuscript. We thank MBL equipment resources, MBL Apparatus Department and Zeiss Microscopes for assistance with equipment. We thank the MBL Central Microscopy facility for providing imaging resources and the Aquatic Resources Division of MBL for supplying squid. We are very grateful for funding from ONR Basic Research Challenge grant # N00014-10-1-0989. Additional funding was also provided by DARPA grant W911NF-10-1-0113 and AFOSR grant FA9950090346.